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1. REPORT DATE (DD-MM-YYYY) 23 May 2017		2. REPORT TYPE Journal Article		3. DATES COVERED (From – To) January 2013 – February 2017	
4. TITLE AND SUBTITLE Operational Mosquito and Vector-Borne Diseases Surveillance at Incirlik Air Base, Turkey				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Will K. Reeves, PhD, MS; Myrna M. Miller, DVM, PhD; Orhan Bayik, PHS; Maj Leah Chapman, USAF, MS				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) USAF School of Aerospace Medicine Public Health and Preventive Medicine Dept/PHR 2510 Fifth St., Bldg. 840 Wright-Patterson AFB, OH 45433-7913				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSORING/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT DISTRIBUTION STATEMENT A. Approved for public release. Distribution is unlimited.					
13. SUPPLEMENTARY NOTES DoD Office of Prepublication & Security Review, Case # 17-S-1077, 8 Mar 2017. US Army Med Dep J. 2017 Jan-Jun; (1-17):86-89					
14. ABSTRACT Arboviruses on Incirlik Air Base, Turkey, pose a threat to military personnel and civilians, but might also be relevant for understanding the threats in neighboring conflict zones such as Syria. We reviewed 6 years of mosquito and arbovirus surveillance at Incirlik Air Base. Over 6,000 were identified as <i>Aedes caspius</i> , <i>Anopheles claviger</i> , <i>Culex mimeticus</i> , <i>Cx. perexiguus</i> , <i>Cx. pipiens</i> , <i>Cx. sinaiticus</i> , and <i>Culiseta longiareolata</i> . Almost all of the mosquitoes (more than 90%) were <i>Cx. perexiguus</i> or <i>Cx. pipiens</i> . Both West Nile virus and Sindbis virus were detected in 6 mosquito pools among collections made in 2013, 2014, and 2015.					
15. SUBJECT TERMS Culicidae, West Nile virus, Sindbis virus, mosquito, Turkey, Incirlik					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON Will Reeves, PhD
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

Operational Mosquito and Vector-Borne Diseases Surveillance at Incirlik Air Base, Turkey

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ABSTRACT

Arboviruses on Incirlik Air Base, Turkey, pose a threat to military personnel and civilians, but might also be relevant for understanding the threats in neighboring conflict zones such as Syria. We reviewed 6 years of mosquito and arbovirus surveillance at Incirlik Air Base. Over 6,000 mosquitoes were identified as *Aedes caspius*, *Anopheles claviger*, *Culex mimeticus*, *Cx. perexiguus*, *Cx. pipiens*, *Cx. sinaiticus*, and *Culiseta longiareolata*. Almost all of the mosquitoes (more than 90%) were *Cx. perexiguus* or *Cx. pipiens*. Both West Nile virus and Sindbis virus were detected in 6 mosquito pools among collections made in 2013, 2014, and 2015.

Vector-borne and zoonotic diseases pose a threat to active military personnel both overseas and in the United States. Deaths among service members from vector-borne diseases have dropped in recent decades, but morbidity from vector-borne diseases and injuries from stinging arthropods remain a real threat.¹⁻⁴ The US Air Force (USAF) maintains arthropod identification and pathogen surveillance capability at the USAF School of Aerospace Medicine at Wright-Patterson Air Force Base, OH, through the Epidemiology Consult Service. Entomology services include the capability to identify both wide range of arthropods from around the world and pathogen surveillance including the detection of several arboviruses, *Bartonella* spp., *Leishmania* spp., *Plasmodium* spp., and *Rickettsia* spp.⁵⁻¹⁰ Many of these identifications and pathogen screening services are used for both threat assessments and as part of standard pest management operations.

Long-term data on both vectors and associated pathogens are maintained for later review and determination of epidemiologic patterns. With the ongoing conflicts in the Middle East, analysis of trends in pathogen surveillance continues to be relevant. Several mosquito-borne arboviruses including West Nile virus (WNV) and Sindbis virus (SINV) were historically reported from both military members and local mosquitoes in the region.^{11,12} We reviewed the arbovirus surveillance data for Incirlik Air Base (AB) in Turkey from 2011-2016 with a discussion of 2 major viruses.

Incirlik AB is in a strategically relevant location. It is located in far southern Turkey relatively near Syria and

other Middle Eastern countries, including Iraq, Lebanon, and Israel. Surveillance is relevant for ongoing force health protection. In addition, pathogens detected in Incirlik could be useful in understanding viruses circulating in neighboring countries. Several mosquito-borne viruses such as West Nile and Sindbis are endemic in Turkey.^{13,14}

METHODS

The US Air Force currently maintains several military bases in the Middle East, but Incirlik AB, which opened in February 1955, has one of the longest established records. Most bases conduct vector and pathogen surveillance programs. Mosquitoes on many of these bases breed in water retention ponds or subsurface catch basins below parking lots or streets. Mosquitoes were trapped on the air base using the solid-state Army miniature traps with dry ice. Traps were set out seasonally from April through October and run on a weekly basis. Mosquito fogging typically occurred 3 days a week. Two traps were used, both near the perimeter fence, one near the sewage treatment plant, and one near base quarters. Mosquitoes were killed by freezing and shipped dry to the USAF School of Aerospace Medicine for identification and pathogen detection. Mosquitoes were morphologically identified using regional keys.^{15,16} Voucher specimens were deposited in the Ohio State University Museum of Biological Diversity, Columbus, OH, or the Walter Reed Biosystematics Unit, Silver Spring, MD.

After identification, mosquitoes were pooled by species and tested for arboviruses. Pools ranged from 1-25 mosquitoes depending on submission numbers. Most

mosquitoes were tested with a RAMP* WNV test and the VecTOR Test Systems (Thousand Oaks, California) *Alphavirus* panel that detects chikungunya (CHIK), equine encephalitis, Mayaro, SINV, Venezuelan equine encephalitis, and Western equine encephalitis viruses. These rapid assays which had been designed for field deployment and rapid screening of mosquitoes¹⁷ were among the tools used from 2011-2016 by the USAF School of Aerospace Medicine. Both the VecTOR Test Systems and RAMP assays were performed in accordance with manufacturers' guidelines. We occasionally used commercially available inactivated West Nile and chikungunya virus antigens as a positive control to validate the assay sensitivity as suggested.¹⁸ As a further validation, we verified 2 of the positive pools by reverse transcription polymerase chain reaction (RT-PCR). West Nile virus positive pools were verified using the protocols by Lanciotti et al.¹⁹ The PCR products were sequenced and compared to sequences in GenBank using the BLAST[†] program.

There were 6 pools of *Culex perexiguus* Theobald or *Cx. pipiens* L. that tested positive for arboviruses as shown in the Table. There were 4 pools positive for WNV: one each on October 2 and 9, 2013; August 29, 2014; and September 16, 2015. We validated the October 9, 2013 results with both the RAMP and VecTOR Test Systems WNV assays. The RAMP scores ranged from 150 to the maximum limit of 740. Both pools tested by RT-PCR were positive with cycle threshold scores of 18.14 and 21.04 respectively which was less than the viral culture positive controls. The 919 base-pair sequence of WNV envelope protein was a 100% match to WNV isolate Spain/2010/H-1b (GenBank # JF719069).

In addition, 2 pools of combined *Cx. perexiguus* and *Cx. pipiens* from June 18 and 25, 2014, tested strongly positive for an *Alphavirus* on the VecTOR Test System assay. This assay is designed to detect specific viruses, and Sindbis is the detectable virus in the region.¹³

COMMENT

We detected West Nile and Sindbis viruses in pools of *Culex* spp. from Incirlik AB. The WNV was in the Western Mediterranean WNV subtype in the WNV lineage 1 based on sequence similarity. While often underappreciated, both viruses are a true threat to the military. Petersen et al²⁰ estimated that WNV caused hundreds of thousands of infections in the United States annually from 1999 to 2010. Further, WNV was the leading cause

of vector-borne disease death in USAF-associated individuals since the 1970s.² Sindbis virus infection causes severe arthritis, fever, and, in rare cases, encephalitis.²¹

West Nile virus was detected in a total of 6 collections made in 2013, 2014, and 2015. This indicates that the virus circulates at some regularity and could pose a threat to both active duty, contractors, civilian personnel, and dependents. We separated mosquito collection data between sewage or waste water treatment plants and residential areas and detected infected mosquitoes in both areas. This virus requires avian hosts for the natural enzootic cycle but mosquitoes can be transovarially infected.²² We could not determine the route of infection in mosquitoes from Incirlik AB.

Incirlik AB is close to Syria and other conflict zones in the Middle East. Detection of 2 potentially serious arbovirus threats on a military base in southern Turkey could raise concerns for similar disease threats in neighboring countries. This is especially true in conflict zones with little to no infrastructure. Infections by either West Nile or Sindbis virus often lead to mild disease but can lead to fatal or debilitating infections and might result in costly medical evacuations.

In summary, we reviewed 6 years (2011-2016) of WNV and SINV detection in mosquitoes from Incirlik AB. *Culex perexiguus* was the most commonly associated mosquito with WNV from the base. It is a member of the *Cx. univittatus* Theobald complex and several of these have previously been associated with both WNV and SINV with evidence of vertical transmission of WNV.²³ Our surveillance data suggests that there are repeated and predictable threats from WNV and possibly SINV on the base.

ACKNOWLEDGEMENTS

We thank the Public Health and Civil Engineering personnel at Incirlik AB for mosquito surveillance and timely submission of samples. We also thank the current and former Entomology staff from the US Air Force School of Aerospace Medicine.

This research was partially funded by the Global Emerging Infections Surveillance and Response System.

This article was cleared by the Defense Office of Pre-publication and Security Review, Security Review# 17-S-1077.

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*RAMP (rapid analyte measurement platform) is a registered trademark of Response Biomedical, Vancouver, Canada.

†Basic Local Alignment Search Tool; National Center for Biotechnology Information, Bethesda, MD.

OPERATIONAL MOSQUITO AND VECTOR-BORNE DISEASES SURVEILLANCE AT INCIRLIK AIR BASE, TURKEY

Mosquito surveillance data from Incirlik Air Base, Turkey, from 2011-2016 with WNV virus and Sindbis virus screening data.			
Locality name	Collection Dates	Species* and Number	Arbovirus Test Results
Housing	2 June-19 August 2011	<i>Culex perexiguus</i> (95) <i>Culex pipiens</i> (23) <i>Aedes caspius</i> (1) <i>Culiseta longiareolata</i> (7)	WNV/Alphavirus Negative
Housing	27 April-14 September 2012	<i>Culex perexiguus</i> (133) <i>Culex pipiens</i> (388) <i>Culex mimeticus</i> (7) <i>Aedes caspius</i> (7) <i>Culiseta longiareolata</i> (6)	WNV/Alphavirus Negative
Running Track	1 June 2012	<i>Culex perexiguus</i> (5) <i>Culex pipiens</i> (13)	WNV/Alphavirus Negative
Housing	18 April-20 November 2013	<i>Culex perexiguus</i> (73) <i>Culex pipiens</i> (339) <i>Culex sinaiticus</i> (148) <i>Aedes caspius</i> (17) <i>Culiseta longiareolata</i> (7)	One pool of 25 <i>Culex perexiguus</i> from 9 October 2013 was positive for WNV virus.
Sewage Treatment Plant	18 April-20 November 2013	<i>Culex perexiguus</i> (821) <i>Culex pipiens</i> (252) <i>Culex sinaiticus</i> (301) <i>Aedes caspius</i> (32)	One pool of 25 <i>Culex perexiguus</i> from 2 October 2013 was positive for WNV virus.
Flight Line	8 April-6 November 2013	<i>Culex pipiens</i> (8)	WNV/Alphavirus Negative
Housing	16 May-29 August 2014	<i>Anopheles claviger</i> (1) <i>Culex perexiguus</i> (658) <i>Culex pipiens</i> (153) <i>Culiseta longiareolata</i> (1)	WNV/Alphavirus Negative
Dining Facility	2 July 2014	<i>Culex perexiguus</i> (1) <i>Culex pipiens</i> (3)	WNV/Alphavirus Negative
Sewage Treatment plant	23 April-29 August 2014	<i>Culex perexiguus</i> (327) <i>Culex pipiens</i> (90) <i>Aedes capensis</i> (9)	Two pools of combined <i>Culex</i> spp. (25 each) from 18 and 25 June 2014 tested positive for <i>Alphavirus</i> . One pool of 25 <i>Culex perexiguus</i> from 29 August 2014 was positive for WNV virus.
Housing	6 May-23 September 2015	<i>Culex perexiguus</i> (331) <i>Culex pipiens</i> (280) <i>Aedes caspius</i> (11) <i>Culiseta longiareolata</i> (14)	One pool of 25 <i>Culex perexiguus</i> from 16 September 2015 was positive for WNV virus.
Sewage Treatment Plant	1 May-16 September 2015	<i>Culex perexiguus</i> (831) <i>Culex pipiens</i> (198) <i>Aedes caspius</i> (2) <i>Culiseta longiareolata</i> (3)	WNV/Alphavirus Negative
Housing	12 April-12 August 2016	<i>Culex perexiguus</i> (11) <i>Culex pipiens</i> (33) <i>Culiseta longiareolata</i> (3)	WNV/Alphavirus Negative
Sewage Treatment Plant	31 March-16 October 2016	<i>Culex perexiguus</i> (225) <i>Culex pipiens</i> (182) <i>Aedes capensis</i> (3)	WNV/Alphavirus Negative
*Scientific species identification: <div style="display: flex; justify-content: space-between;"> <div> <i>Aedes capensis</i> Edwards <i>Aedes caspius</i> (Pallas) <i>Culiseta longiareolata</i> Macquart <i>Culex mimeticus</i> Noè </div> <div> <i>Culex perexiguus</i> Theobald <i>Culex pipiens</i> L. <i>Culex sinaiticus</i> Kirkpatrick </div> </div>			

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